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Specification and Drawings, as originally filed, with Application for Patent Serial No:**2,399,548** on August 23, 2002, by **UNIVERSITÉ DE MONTRÉAL**, assignee of Huy Ong, Sylvie Marleau and André Tremblay, for "Growth Hormone-Releasing Peptides as Negative Modulators of Atherosclerosis and Hypercholesterolemia".

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July 21, 2003
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ABSTRACT OF THE DISCLOSURE

Growth hormone releasing peptides (GHRPs), a family of synthetic analogs modelled from Met-enkephalin, have been found to bind a novel class of specific GHRP receptors in the mammalian heart that are distinct from the pituitary GHRP receptors involved in GH secretion. CD36, a multifunctional B-type scavenger receptor, has been identified as the unique GHRP binding site in the heart. CD36 mediates the uptake of lipoproteins into a number of cell types and was recognized to play a crucial role in scavenging oxidized low density lipoproteins in monocyte/ macrophages leading to foam cell formation, a key step in fatty streaks formation.

The hexapeptide GHRP prototypes hexarelin (His - DMe – Trp – Ala – Trp – D Phe – Lys - NH_2) and EP 80317 (Haic-D Me - Trp –D Lys - Trp - D Phe- Lys - NH_2), the latter analog being devoid of GH-secreting activity *in vivo*, have been shown to reduce fatty streak lesions and hypercholesterolemia in apolipoprotein E (Apo E) null mice fed a high fat high cholesterol diet (HFHC). In addition, non-HDL cholesterol was significantly decreased, whereas HDL cholesterol tended to increase. GHRP treatment also inhibited oxLDL-induced monocyte chemotaxis in the peritoneal cavity, which was associated with a reduced expression of the CD36 protein on peritoneal monocytes/macrophages.

GHRPs may represent a novel therapeutic avenue to reduce the severe morbidity associated with the arterial disease atherosclerosis and its complications, such as myocardial infarction and strokes, that occur despite current management strategies.

TITLE OF THE INVENTION

GROWTH HORMONE-RELEASING PEPTIDES AS NEGATIVE MODULATORS OF ATHEROSCLEROSIS AND HYPERCHOLESTEROLEMIA

SUMMARY OF THE INVENTION

Atherosclerosis is a multifactorial disease developing preferentially in subjects presenting biochemical risks factors including smoking, hypertension, diabetes mellitus, hypercholesterolemia, elevated plasma low density lipoprotein (LDL) and triglycerides, hyperfibrinogenemia and hyperglycemia, among others. Atherosclerotic lesions develop over a number of decades in humans, leading to complications such as coronary and cerebral ischemic and thromboembolic diseases and myocardial and cerebral infarction. To date, cardiovascular disease is the leading cause of morbidity and mortality in industrialized countries and progresses steadily in emerging countries, with coronary atherosclerosis being the main underlying pathology (1-3). Currently, therapy of atherosclerosis is not completely efficient to prevent disease development and complication.

Atherosclerosis develops through the sequential interplay of at least 3 pathological processes: foam cell differentiation, inflammatory reaction and cell proliferation. Key players in these processes are the injured endothelium, monocytes/macrophages and smooth muscle cells, and a regulatory network of growth factors and cytokines (4). One of the earliest detectable events in the development of early fatty streak lesions in human and various animal models is the recruitment of mononuclear phagocytes and lymphocytes to the intact endothelial lining of large arteries (4). Enhanced adhesion and accumulation of blood monocytes into the intima is then accompanied by a change in cell phenotype, where they transform into macrophages. The latter engulfs lipids and stores them as cytoplasmic droplets, thus becoming «foam cells». Macrophage-derived-foam cells orchestrate events (together with T-lymphocytes) leading to lesion progression and smooth muscle cell (SMC) accumulation into the intima. SMCs also adopt a dedifferentiated synthetic phenotype (as opposed to their differentiated contractile phenotype), proliferate and produce cytokines and proteinases favoring lesion development. Hence, accumulation of mononuclear cells in arterial-prone sites is a central inflammatory event in atherogenesis.

Oxidative stress induces macrophage (and to a lesser extent monocytes) lipid peroxidation and cellular accumulation of oxidized lipids and cholesterol products (oxysterols) (5). In return, oxidized macrophages induce oxidation of LDL, and minimally and fully oxidized (mm- or ox-) forms of LDL are found (6). OxLDL have been shown to induce monocyte and SMC chemotaxis (while inhibiting macrophage motility) and

stimulate macrophage and SMC growth. OxLDL has been recognized as a major determinant in the initiation of fatty streak lesions and in their progression (7-10). By being internalized in macrophages, oxLDL provide a source of oxidized fatty acids and oxysterols that serve as endogenous ligands for the activation of nuclear receptors PPAR (peroxisome proliferator-activated receptor) and LXR (liver X receptor). PPAR and LXR are considered critical regulators in the expression of genes involved in various steps controlling cholesterol homeostasis. In particular, PPARγ isoform was shown to regulate CD36 expression and therefore macrophage oxLDL uptake in a positive feedback mechanism(11-13). Other components involved in cellular cholesterol mobilization and efflux from macrophages, such as the cholesterol 27-hydroxylase CYP27, the apolipoprotein E, and the ATP-binding cassette ABCA1 transporter, were shown to be upregulated by LXR, which is by itself a known target for PPARγ. In this regard, the PPARγ-LXRα-ABCA1 regulatory cascade that coordinates oxLDL-derived cholesterol uptake, processing and removal from macrophages has been proposed as a physiological target for developing potential therapeutic avenues to maximally reduce plaque burden (14;15).

Growth hormone releasing peptides (GHRPs), which consist of a family of small synthetic peptides modelled from Met-enkephalin are reported to feature potent and dose dependent growth hormone-releasing activity and significant prolactin and corticotropin–releasing effects (16). These neuroendocrine activities of GHRPs are mediated by a specific G protein–coupled receptor identified as Ghrelin receptor expressed in hypothalamus and pituitary gland (17). In addition, these peptides appear to feature cardioprotective effect against cardiac ischemia in growth hormone (GH) deficient or aged rats (18) (19). This protective activity is not coupled to any apparent stimulation of the somatotropic function, suggesting a direct myocardial action of these peptides (18). In documenting the distribution of GHRP binding sites in the cardiovascular system by covalent photoaffinity labelling approach, we have uncovered that hexarelin, a hexapeptide member of the GHRPs family, binds to a glycosylated membrane protein of 84 kD distinct from the Ghrelin receptor which was identified as CD36, a type B scavenger receptor (20) (21). This scavenger receptor, known initially as serum long-chain fatty acids transporter, mediates the uptake of lipoproteins into a number of cells such as adipocytes, myocytes and monocytes/macrophages (22). CD36 is also recognized to play a key role in scavenging oxidized low density lipoproteins into monocytes/macrophages, a process that leads to foam cell formation and initiation of fatty streak development (7;23;24).

The present invention relates to the use of GHRPs and related synthetic analogs of the hexapeptide prototype hexarelin (His - DMe – Trp – Ala – Trp – D Phe – Lys - NH_2)

and of the prototype EP 80317 (Haic-D Me - Trp –D Lys - Trp - D Phe- Lys - NH2), which is a GHRP analog devoid of GH-secreting activity *in vivo*, and of any ligand which binds to their receptor(s), in the treatment or prophylaxis of cardiovascular diseases associated with atherosclerosis in humans and animals.

The present inventors have found the existence of a new class of specific GHRP receptors in the mammalian heart, distinct from the pituitary GHRP receptors involved in GH secretion (20). The present inventors have identified CD36, a multifunctional B-type scavenger receptor, as the unique GHRP binding site in the heart (21).

The present inventors have found that a prolonged treatment (12 weeks) with GHRP interferes with the scavenger receptor function and expression, thereby reducing the uptake of oxLDL and the accumulation of lipids and cholesterol, and consequently, reducing the formation of fatty streak lesions in ApoE null mice. The ApoE null mouse has been selected as an experimental model of atherosclerosis as it features the progressive series of atherogenic events seen in human, including increased adhesive interactions between leukocyte and endothelium, conversion of monocyte-derived macrophages into foam cells with lesions distributing throughout the arterial tree, and late development of more advanced lesions (fibrous plaques). ApoE null mice show very high levels of plasma cholesterol as a result of impaired clearance of cholesterol-enriched lipoproteins and, as for humans, high fat high cholesterol (HCHF) diet exacerbates disease progression and markedly enhances plasma cholesterol levels (7) (25). Evidence accumulates to support that fatty streaks in anatomical sites prone to atheromatous plaque development precede mature lesions in humans, although all fatty streaks do not progress to atheromas (26). In both human and mice, T lymphocyte and foam cells are found in fatty streaks and immunological processes have been shown to be similar over the years in both species.

The present inventors have found that HEX, as well as with EP80317, reduced total plasma cholesterol and non-HDL cholesterol, and increased HDL cholesterol in ApoE-deficient mice fed with a HFHC diet from 6 weeks old. Hence, favorable changes in plasma lipids were associated with a significant decrease in fatty streak lesion area in ApoE null mice treated with GHRPs as compared with controls.

Furthermore, the present inventors found that GHRPs regulated the expression of the CD36 protein in peritoneal macrophages from ApoE null mice in mice fed a HFHC diet for 12 weeks.

The present inventors have also found using cultured macrophages from differentiated monocytic THP-1 cells, that treatment with HEX, as well as with EP80317, contributed to decrease lipid storage in macrophages, with a concomitant increase in gene expression of nuclear receptor LXRα and ABCA1 transporter, two proteins involved in cellular cholesterol efflux.

Hence, GHRPs analogs might prevent cholesterol accumulation in the macrophages and foam cell formation, thereby reducing fatty streak development. Thus, GHRPs may be efficient to prevent the development of atherosclerosis plaques and cardiovascular disease linked to atherosclerotic processes such as coronary artery disease, myocardial infarction and strokes.

To our knowledge, no other drug shares the mechanism of action of GHRPs and, therefore, GHRPs represent a novel therapeutic avenue for the treatment or prophylaxis of atherosclerosis and associated diseases.

Therefore, in accordance with the present invention is provided a method for preventing fatty streaks formation and atherosclerosis development through reducing CD36 expression which leads to inhibition of oxLDL internalization, thus breaking the cycle of foam cell formation and the feed-forward loop of oxLDL-induced PPARγ and CD36 expression (12), in addition to reducing total plasma cholesterol and non HDL cholesterol. The steps include systemic administration of GHRP to afford a protective amount of drug in circulation.

The GHRPs prototypes tested are Hexarelin and EP80317.

In a most specific embodiment, Hexarelin, EP 80317 and any GHRP analog is exogenously administered.

This invention will be described herein below, referring to specific embodied exemples and appended figures, which purpose is to illustrate the invention rather than limit its scope.

BRIEF DESCRIPTION OF THE DRAWINGS

Having thus generally described the invention, reference will now be made to the accompanying drawings, showing by way of illustration a preferred embodiment thereof, and in which:

Figure 1

GHRPs prevent fatty streak formation in ApoE deficient mice fed a HFHC diet for 12 weeks. Effects of HEX (100 µg/kg per day) and of EP80317 (300 µg/kg per day), administered subcutaneously for 12 weeks. A. Lesion area (% of total aorta area) on ApoE null aortas were reduced by 28 and 47% following treatment with HEX and EP80317, respectively, as compared to controls treated with 0.9% NaCl. The open bar represents vehicle (0.9% NaCl) treated mice, the solid bar, HEX treatment and the cross-hatched bar, EP80317 treatment. Asterisk indicates $P < 0.05$ (**, $P < 0.01$) compared with vehicle, and # indicates $P < 0.05$ compared with HEX. B. Photograph of representative mice aortas from vehicle- (top), Hex- (middle) and EP80317-treated (bottom) ApoE null mice stained with oil red O.

Figure 2

GHRPs reduced total plasma cholesterol and non HDL cholesterol, and tended to increase HDL cholesterol in ApoE null mice fed a HFHC diet for 12 weeks. Total plasma cholesterol and nonHDL cholesterol were decreased by 30 and 31%, respectively, in EP80317-treated ApoE null mice under HFHC diet as compared to controls. HDL cholesterol increased by 65 and 73% in ApoE null mice under HFHC treated with HEX and EP80317, respectively. A. Effects of 12 weeks treatment (from 6 weeks old) with 0.9% NaCl (open bar), HEX, 100 µg/kg per day (solid bar) or EP80317 300 µg/kg per day (cross-hatched bar) on total plasma cholesterol in mice fed a HFHC or a normal diet. B. Effects of treatments on plasma triglycerides. C. Effects of treatments on HDL cholesterol. D. Effects of treatments on non-HDL cholesterol.

Asterisk indicates $P < 0.01$ compared with vehicle, and # indicates $P < 0.01$ compared with HEX.

Figure 3

GHRPs reduced the accumulation of oxLDL-induced peritoneal macrophage accumulation in wild type and in ApoE null mice fed a HFHC diet for 12 weeks. A. Effects of 12 weeks treatment (from 6 weeks old) with 0.9% NaCl (open bar), HEX, 100 µg/kg per day (solid bar) or EP80317 300 µg/kg per day (cross-hatched bar) on oxLDL (250 µg i.p., < 6 nmol MDA/mg lipoprotein)-induced accumulation of macrophages in the peritoneal cavity in wild type C57BL/6 mice and CD36 null mice fed a HFHC diet. Peritoneal macrophage accumulation in wild type mice tended to be reduced by 37%. B. Effects of 12 weeks treatment with 0.9% NaCl (open bar), HEX, 100 µg/kg per day (solid bar) or EP80317 300 µg/kg per day (cross-hatched bar) on oxLDL-induced accumulation of macrophages in the peritoneal cavity in ApoE null mice fed either a HFHC or a normal diet. Peritoneal macrophage accumulation was reduced by 39% in mice fed a normal diet.

Figure 4

GHRPs reduced oxLDL-induced expression of CD36 in mouse macrophages in ApoE null mice fed a HFHC diet for 12 weeks. Effects of 12 weeks treatment (from 6 weeks old) with 0.9% NaCl (open bar), HEX, 100 µg/kg per day (solid bar) or EP80317 300 µg/kg per day (cross-hatched bar) on oxLDL (250 µg i.p., < 6 nmol MDA/mg lipoprotein)-induced accumulation of macrophages in the peritoneal cavity in wild type C57BL/6 mice and CD36 null mice fed a HFHC diet. Peritoneal macrophage were harvested and 1×10^6 macrophages were assayed for CD36 protein level determination by Western blot. CD36 protein was reduced to 57 and 27% of controls, in macrophages from HEX and EP 80317-treated mice, respectively.

Figure 5

GHRPs did not modulate the growth curve in ApoE null mice fed a HFHC diet (or a normal diet) for 12 weeks. A. Effects of 12 weeks treatment (from 6 weeks old) with 0.9% NaCl (diamond), HEX, 100 µg/kg per day (square) or EP80317 300 µg/kg per day (triangle) on weight of in mice fed a HFHC or B, a normal diet.

Figure 6

GHRPs did not modulate food intake in ApoE null mice fed a HFHC diet (or a normal diet) for 12 weeks. A. Effects of 12 weeks treatment (from 6 weeks old) with 0.9% NaCl (diamond), HEX, 100 µg/kg per day (square) or EP80317 300 µg/kg per day (triangle) on food intake in mice fed a HFHC or B, a normal diet.

Figure 7

A. GHRPs reduce lipid accumulation in differentiated human macrophages. To induce lipid accumulation, human monocytic THP-1 cells were differentiated to macrophages with 5ng/ml PMA for 48hrs and then treated as indicated for 24hrs with 10uM HEX, or EP80317 in the presence of PMA. Non-differentiated cells (no PMA) were also analyzed. Lipid accumulation was quantified by photometry at 510nm following extraction of Oil red O from stained cells. Lipid staining was reduced by 35% and 28% in macrophages treated with HEX and EP80317, respectively. B. GHRPs reduced lipid accumulation in peritoneal macrophages isolated from ApoE null mice fed a HFHC diet from 13 weeks old. EP80317 (300 µg/kg) s.c. has been administered from 13 to 18 weeks old. Lipid staining (assessed by Oil red O extraction) was reduced by 57%. Figure 8

GHRPs increase expression of genes involved in cellular cholesterol removal.

A, PMA-differentiated THP-1 macrophages were treated with HEX or EP80317 as described in Figure 7, and mRNA expression was analyzed for selected genes by RT-PCR. The procedure involves extraction of total RNA from cells which is then reverse transcribed into cDNA and amplified in a PCR reaction with specific primers. Primers used are:

LXRα forward CCTGTCAGAAGAACAGATCCGC

LXRα reverse TCTTCAGCAGGGCAATCTGGTCC;

ABCA1 forward GGTCAATGGAAGGTTCAGGTGC

ABCA1 reverse GGAGTCGCTTTTTGCTCTGGGAGAGG;

GAPDH forward GGTCTTACTCCTTGGAGGCCATGT

GAPDH reverse GACCCCTTCATTGACCTCAACTACA.

B, Measurement of signal intensity from the experiment described in A was performed using an Alpha Imager analysis system and results expressed as fold response compared to untreated differentiated cells. GAPDH was used as a control for data normalization.

EXAMPLE 1

Growth hormone-releasing peptides prevent fatty streaks formation

Severe morbidity results as a consequence of the arterial disease atherosclerosis and complications of the disease, such as myocardial infarction and strokes, remain a common cause of mortality in Western Society (27). Current management strategies of atherosclerosis include life-style interventions such as healthy dietary and exercise recommendations and, mainly, the use of lipid lowering drug therapy, in addition to blood pressure control and use of antiplatelet drugs. Among hypolipemic drugs, hepatic hydroxymethylglutaryl-coenzyme A reductase inhibitors or statin drugs interfere with hepatic cholesterol metabolism which leads to a compensatory increase in LDL receptors and cholesterol clearance via these receptors. In contrast, fibrates and nicotinic acid mainly reduce circulating VLDL whereas bile acid binding resins interfere with bile acid reabsorption, thereby increasing fecal excretion of cholesterol. However, statins, used in monotherapy or in association with another lipid-lowering drug, have been associated with serious adverse effects, myopathy and rhabdomyolisis (28). For instance, cerivastatin therapy has been associated with 100s of myopathies and some dozens of deaths (29). Hence, use of these drugs should be revisited (30). In contrast, the mechanism of action of GHRPs, although not completely elucidated, is likely to involve the function of CD36 and its expression.

To determine whether GHRPs reduces atherosclerosis development, we have used the ApoE deficient mice strain and their C57BL/6 control littermates to assess the effects of prolonged (12 weeks) GHRPs treatment on fatty streak formation in mice fed a an enriched lipid diet. The surface area of oil red-O staining aortas of mice has been used as an index of plaque development and changes in the plasma levels of lipids as an index of hypolipemic effect. Taken together, these end points served to evaluate the anti-atherosclerotic effect GHRPs *in vivo*.

Methods

Drugs

HEX and EP80317 were a generous gift of Dr. R. Deghenghi, Europeptides, Argenteuil, France. HEX and EP80317 stock solutions were prepared in sterile 0.9% NaCl.

Animals

CD36-deficient and ApoE-deficient mice, as well as their control littermate were raised in the animal facilities of the Université de Montréal. The ApoE deficient mouse features the progressive series of atherogenic events seen in human, including increased adhesive interactions between leukocyte and endothelium, conversion of monocyte-

derived macrophages into foam cells with lesions distributing throughout the arterial tree, and late development of more advanced lesions (fibrous plaques). ApoE null mice show very high levels of plasma cholesterol as a result of impaired clearance of cholesterol-enriched lipoproteins and, as for humans, HCHF diet exacerbates disease progression and markedly enhances plasma cholesterol levels (7) (25). However, complex lesions as seen in humans are not observed except for the innominate artery in mice ~ 42 weeks old, where loss of continuity of the fibrous cap, rupture of xanthomas at the shoulders of lesions and intraplaque haemorrhage are seen (31). Evidence accumulates to support that fatty streaks in anatomical sites prone to atheromatous plaque development precede mature lesions in humans, although all fatty streak do not progress to atheromas (26). In both human and mice, T lymphocyte and foam cells are found in fatty streaks and immunological processes have been shown to be similar over the years in both species.

The animals were housed in cages (less than 5 per cage) and fed a normal chow diet and water ad libitum. Male C57BL/6, CD36 and ApoE null mice were assigned to 1 of 3 groups (n=12 mice per group): Group 1 received daily injections of HEX (100 µg/kg, 1 µl/g); group 2, EP 80 317 (300 µg/kg, 1 µl/g), and group 3, 0.9% NaCl. Daily s.c. treatment with HEX, EP80317 or vehicle was begun at 6 weeks old and continued for 12 weeks. Four days before sacrifice, 6 mice from groups 1 to 3 were injected i.p. with oxLDL (minimally oxidized, TBARS 3-6 nmol/mg protein). At 18 weeks old, mice were fasted overnight, anesthetized with ketamine-xylazine (90:10 mg/kg) two hours after the s.c. administration of the morning dose of the drug under study. Blood (1 ml) was taken from the heart and put into EDTA pre-coated microcontainers (BD, Franklin Lakes, NJ, USA). Mice were killed, the hearts were perfused with 20 ml 0.9% NaCl. The peritoneal cavity was washed with 3 ml of heparinized saline (10 units/ml) in mice injected i.p. with oxLDL. A hemacytometer and stained cytospin preparation (Diff Quick stain, Dade Diagnostics of P.R. Inc., Aguada, PR) were used to determine the total and differential leukocyte numbers, respectively, for the peritoneal cavity lavage fluid. The entire aorta from the heart, extending 5-10 mm after bifurcation of the iliac arteries and including the subclavian right and left common carotid arteries, was removed, dissected, and evaluated for lesion development by en face oil red-O staining and morphometry of scanned images using the software Scion Image (Scion Corp., Fredrick, Maryland). The animal study protocol was reviewed and approved by the institutional Animal Ethics Committee of the Université de Montréal and conducted in accordance with the Canadian Council on Animal Care guidelines for use of experimental animals.

Plasma lipid analysis

Total plasma cholesterol, triacylglycerol and HDL cholesterol were determined using enzymatic kits (Sigma Chemicals). Appropriate standards and controls were included in each assay.

Statistical analysis

Data are expressed as mean ± SEM. Comparisons between groups were performed using a one-way analysis of variance (ANOVA) followed by pair-wise multiple comparisons using the Student-Newman-Keuls method. Differences were considered significant at $p < 0.05$.

Results

The most striking observation is a reduction of lesions area in ApoE-deficient mice, by 28 and 47% following treatment with HEX (100 µg/kg) and EP80317 (300 µg/kg) daily, respectively, in mice fed a HFHC diet (fig 1). CD36-deficient mice and their wild type C57BL/6 control littermates did not develop significant fatty streak lesions on HFHC diet (12 weeks).

Reduced lesions area was accompanied with a decrease in total plasma cholesterol (30%), as well as in non HDL plasma cholesterol (31%), in ApoE-deficient mice fed a HFHC diet and treated daily with EP80317 (300 µg/kg) daily for 12 weeks, as compared to controls and to ApoE null mice treated treated with HEX (100 µg/kg) daily (Fig 2A and Fig 2C). In contrast, HDL cholesterol tended to be increased in ApoE null mice under lipid diet (12 weeks) treated with HEX (65%) or EP80317 (73%) as compared to controls on HFHC diet (Fig 2D). Plasma triglycerides did not change significantly (Fig 2B). GHRPs did not modulate total plasma cholesterol levels in CD36-deficient or C57BL/6 controls fed a HFHC diet (data not shown).

EP80317 reduced oxLDL-induced peritoneal macrophage accumulation by 37% and 39 % in wild type C57BL/6 and ApoE null mice, respectively (Fig 3A and Fig. 3B). EP 80317 did not modulate macrophage accumulation in CD36 null mice (Fig 3A). CD36 protein expression was reduced to 57 and 27% of controls, in peritoneal macrophages harvested from HEX and EP 80317-treated ApoE null mice fed a HFHC diet, respectively (Fig. 4).

GHRPs therapy did not affect the growth curve in ApoE-deficient mice fed a HFHC diet (Fig 5A) or a normal diet (Fig 5B), nor the food intake in these mice (Fig 6A and Fig 6B).

Discussion

In the present study, we have assessed the contribution of GHRPs in the protective effect against atherosclerosis development. The major findings are 1) that a prolonged treatment with GHRPs protects mice fed a HFHC diet from developing fatty

streak lesions 2) these protective effects of GHRPs are associated with a favourable modulation in plasma lipids, inasmuch as total plasma cholesterol and non HDL cholesterol are reduced, and HDL plasma cholesterol is increased, in ApoE null mice fed a HFHC diet 3) the atheroprotective effects of GHRPs are associated with a reduced expression of CD36 protein in macrophages. These findings suggest the possibility that GHRPs therapy, through reducing atherosclerosis development, may afford protection against heart attacks and strokes. Thus, GHRP and their synthetic analogs would appear to be potentially helpful for the treatment or prophylaxis of coronary cardiovascular diseases. For example, they will be useful to prevent hypercholesterolemia and atherosclerosis, thereby reducing the complications associated with the disease. Currently, only few drugs have the capacity to achieve these goals, and these have been associated with potentially severe adverse effects.

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WHAT IS CLAIMED IS:

1. The use of growth hormone releasing peptides of Hexarelin family, of derived peptidomimetics and of CD36 ligands in the prevention and treatment of atherosclerosis and hypercholesterolemia.

2. The use of GHRP derivatives, of derived peptidomimetics, and of CD36 ligands which modulate the expression of scavenger receptor B (CD36) in the development of atherosclerotic lesions and in the prevention of heart attacks and strokes associated with coronary artery disease and hypercholesterolemia.

3. The use of GHRP derivatives and of derived peptidomimetics which modulate the expression of the ATP-binding cassette ABCA1 transporter scavenger receptor B (CD36) in the development of atherosclerotic lesions and in the prevention of heart attacks and strokes associated with coronary artery disease and hypercholesterolemia.

4. A pharmaceutical composition containing a compound as claimed in claims 1, 2 and 3, to be administered exogenously.

TITLE OF THE INVENTION

GROWTH HORMONE-RELEASING PEPTIDES AS NEGATIVE MODULATORS OF ATHEROSCLEROSIS AND HYPERCHOLESTEROLEMIA

SUMMARY OF THE INVENTION

Atherosclerosis is a multifactorial disease developing preferentially in subjects presenting biochemical risks factors including smoking, hypertension, diabetes mellitus, hypercholesterolemia, elevated plasma low density lipoprotein (LDL) and triglycerides, hyperfibrinogenemia and hyperglycemia, among others. Atherosclerotic lesions develop over a number of decades in humans, leading to complications such as coronary and cerebral ischemic and thromboembolic diseases and myocardial and cerebral infarction. To date, cardiovascular disease is the leading cause of morbidity and mortality in industrialized countries and progresses steadily in emerging countries, with coronary atherosclerosis being the main underlying pathology (1-3). Currently, therapy of atherosclerosis is not completely efficient to prevent disease development and complication.

Atherosclerosis develops through the sequential interplay of at least 3 pathological processes: foam cell differentiation, inflammatory reaction and cell proliferation. Key players in these processes are the injured endothelium, monocytes/macrophages and smooth muscle cells, and a regulatory network of growth factors and cytokines (4). One of the earliest detectable events in the development of early fatty streak lesions in human and various animal models is the recruitment of mononuclear phagocytes and lymphocytes to the intact endothelial lining of large arteries (4). Enhanced adhesion and accumulation of blood monocytes into the intima is then accompanied by a change in cell phenotype, where they transform into macrophages. The latter engulfs lipids and stores them as cytoplasmic droplets, thus becoming «foam cells». Macrophage-derived-foam cells orchestrate events (together with T-lymphocytes) leading to lesion progression and smooth muscle cell (SMC) accumulation into the intima. SMCs also adopt a dedifferentiated synthetic phenotype (as opposed to their differentiated contractile phenotype), proliferate and produce cytokines and proteinases favoring lesion development. Hence, accumulation of mononuclear cells in arterial-prone sites is a central inflammatory event in atherogenesis.

Oxidative stress induces macrophage (and to a lesser extent monocytes) lipid peroxidation and cellular accumulation of oxidized lipids and cholesterol products (oxysterols) (5). In return, oxidized macrophages induce oxidation of LDL, and minimally and fully oxidized (mm- or ox-) forms of LDL are found (6). OxLDL have been shown to induce monocyte and SMC chemotaxis (while inhibiting macrophage motility) and

stimulate macrophage and SMC growth. OxLDL has been recognized as a major determinant in the initiation of fatty streak lesions and in their progression (7-10). By being internalized in macrophages, oxLDL provide a source of oxidized fatty acids and oxysterols that serve as endogenous ligands for the activation of nuclear receptors PPAR (peroxisome proliferator-activated receptor) and LXR (liver X receptor). PPAR and LXR are considered critical regulators in the expression of genes involved in various steps controlling cholesterol homeostasis. In particular, PPARγ isoform was shown to regulate CD36 expression and therefore macrophage oxLDL uptake in a positive feedback mechanism(11-13). Other components involved in cellular cholesterol mobilization and efflux from macrophages, such as the cholesterol 27-hydroxylase CYP27, the apolipoprotein E, and the ATP-binding cassette ABCA1 transporter, were shown to be upregulated by LXR, which is by itself a known target for PPARγ. In this regard, the PPARγ-LXRα-ABCA1 regulatory cascade that coordinates oxLDL-derived cholesterol uptake, processing and removal from macrophages has been proposed as a physiological target for developing potential therapeutic avenues to maximally reduce plaque burden (14;15).

Growth hormone releasing peptides (GHRPs), which consist of a family of small synthetic peptides modelled from Met-enkephalin are reported to feature potent and dose dependent growth hormone-releasing activity and significant prolactin and corticotropin–releasing effects (16). These neuroendocrine activities of GHRPs are mediated by a specific G protein–coupled receptor identified as Ghrelin receptor expressed in hypothalamus and pituitary gland (17). In addition, these peptides appear to feature cardioprotective effect against cardiac ischemia in growth hormone (GH) deficient or aged rats (18) (19). This protective activity is not coupled to any apparent stimulation of the somatotropic function, suggesting a direct myocardial action of these peptides (18). In documenting the distribution of GHRP binding sites in the cardiovascular system by covalent photoaffinity labelling approach, we have uncovered that hexarelin, a hexapeptide member of the GHRPs family, binds to a glycosylated membrane protein of 84 kD distinct from the Ghrelin receptor which was identified as CD36, a type B scavenger receptor (20) (21). This scavenger receptor, known initially as serum long-chain fatty acids transporter, mediates the uptake of lipoproteins into a number of cells such as adipocytes, myocytes and monocytes/macrophages (22). CD36 is also recognized to play a key role in scavenging oxidized low density lipoproteins into monocytes/macrophages, a process that leads to foam cell formation and initiation of fatty streak development (7;23;24).

The present invention relates to the use of GHRPs and related synthetic analogs of the hexapeptide prototype hexarelin (His - DMe – Trp – Ala – Trp – D Phe – Lys - NH_2)

and of the prototype EP 80317 (Haic-D Me - Trp –D Lys - Trp - D Phe- Lys - NH2), which is a GHRP analog devoid of GH-secreting activity *in vivo*, and of any ligand which binds to their receptor(s), in the treatment or prophylaxis of cardiovascular diseases associated with atherosclerosis in humans and animals.

The present inventors have found the existence of a new class of specific GHRP receptors in the mammalian heart, distinct from the pituitary GHRP receptors involved in GH secretion (20). The present inventors have identified CD36, a multifunctional B-type scavenger receptor, as the unique GHRP binding site in the heart (21).

The present inventors have found that a prolonged treatment (12 weeks) with GHRP interferes with the scavenger receptor function and expression, thereby reducing the uptake of oxLDL and the accumulation of lipids and cholesterol, and consequently, reducing the formation of fatty streak lesions in ApoE null mice. The ApoE null mouse has been selected as an experimental model of atherosclerosis as it features the progressive series of atherogenic events seen in human, including increased adhesive interactions between leukocyte and endothelium, conversion of monocyte-derived macrophages into foam cells with lesions distributing throughout the arterial tree, and late development of more advanced lesions (fibrous plaques). ApoE null mice show very high levels of plasma cholesterol as a result of impaired clearance of cholesterol-enriched lipoproteins and, as for humans, high fat high cholesterol (HCHF) diet exacerbates disease progression and markedly enhances plasma cholesterol levels (7) (25). Evidence accumulates to support that fatty streaks in anatomical sites prone to atheromatous plaque development precede mature lesions in humans, although all fatty streaks do not progress to atheromas (26). In both human and mice, T lymphocyte and foam cells are found in fatty streaks and immunological processes have been shown to be similar over the years in both species.

The present inventors have found that HEX, as well as with EP80317, reduced total plasma cholesterol and non-HDL cholesterol, and increased HDL cholesterol in ApoE-deficient mice fed with a HFHC diet from 6 weeks old. Hence, favorable changes in plasma lipids were associated with a significant decrease in fatty streak lesion area in ApoE null mice treated with GHRPs as compared with controls.

Furthermore, the present inventors found that GHRPs regulated the expression of the CD36 protein in peritoneal macrophages from ApoE null mice in mice fed a HFHC diet for 12 weeks.

The present inventors have also found using cultured macrophages from differentiated monocytic THP-1 cells, that treatment with HEX, as well as with EP80317, contributed to decrease lipid storage in macrophages, with a concomitant increase in gene expression of nuclear receptor LXRα and ABCA1 transporter, two proteins involved in cellular cholesterol efflux.

Hence, GHRPs analogs might prevent cholesterol accumulation in the macrophages and foam cell formation, thereby reducing fatty streak development. Thus, GHRPs may be efficient to prevent the development of atherosclerosis plaques and cardiovascular disease linked to atherosclerotic processes such as coronary artery disease, myocardial infarction and strokes.

To our knowledge, no other drug shares the mechanism of action of GHRPs and, therefore, GHRPs represent a novel therapeutic avenue for the treatment or prophylaxis of atherosclerosis and associated diseases.

Therefore, in accordance with the present invention is provided a method for preventing fatty streaks formation and atherosclerosis development through reducing CD36 expression which leads to inhibition of oxLDL internalization, thus breaking the cycle of foam cell formation and the feed-forward loop of oxLDL-induced PPARγ and CD36 expression (12), in addition to reducing total plasma cholesterol and non HDL cholesterol. The steps include systemic administration of GHRP to afford a protective amount of drug in circulation.

The GHRPs prototypes tested are Hexarelin and EP80317.

In a most specific embodiment, Hexarelin, EP 80317 and any GHRP analog is exogenously administered.

This invention will be described herein below, referring to specific embodied exemples and appended figures, which purpose is to illustrate the invention rather than limit its scope.

BRIEF DESCRIPTION OF THE DRAWINGS

Having thus generally described the invention, reference will now be made to the accompanying drawings, showing by way of illustration a preferred embodiment thereof, and in which:

Figure 1

GHRPs prevent fatty streak formation in ApoE deficient mice fed a HFHC diet for 12 weeks. Effects of HEX (100 µg/kg per day) and of EP80317 (300 µg/kg per day), administered subcutaneously for 12 weeks. A. Lesion area (% of total aorta area) on ApoE null aortas were reduced by 28 and 47% following treatment with HEX and EP80317, respectively, as compared to controls treated with 0.9% NaCl. The open bar represents vehicle (0.9% NaCl) treated mice, the solid bar, HEX treatment and the cross-hatched bar, EP80317 treatment. Asterisk indicates $P < 0.05$ (**, $P < 0.01$) compared with vehicle, and # indicates $P < 0.05$ compared with HEX. B. Photograph of representative mice aortas from vehicle- (top), Hex- (middle) and EP80317-treated (bottom) ApoE null mice stained with oil red O.

Figure 2

GHRPs reduced total plasma cholesterol and non HDL cholesterol, and tended to increase HDL cholesterol in ApoE null mice fed a HFHC diet for 12 weeks. Total plasma cholesterol and nonHDL cholesterol were decreased by 30 and 31%, respectively, in EP80317-treated ApoE null mice under HFHC diet as compared to controls. HDL cholesterol increased by 65 and 73% in ApoE null mice under HFHC treated with HEX and EP80317, respectively. A. Effects of 12 weeks treatment (from 6 weeks old) with 0.9% NaCl (open bar), HEX, 100 µg/kg per day (solid bar) or EP80317 300 µg/kg per day (cross-hatched bar) on total plasma cholesterol in mice fed a HFHC or a normal diet. B. Effects of treatments on plasma triglycerides. C. Effects of treatments on HDL cholesterol. D. Effects of treatments on non-HDL cholesterol.

Asterisk indicates $P < 0.01$ compared with vehicle, and # indicates $P < 0.01$ compared with HEX.

Figure 3

GHRPs reduced the accumulation of oxLDL-induced peritoneal macrophage accumulation in wild type and in ApoE null mice fed a HFHC diet for 12 weeks. A. Effects of 12 weeks treatment (from 6 weeks old) with 0.9% NaCl (open bar), HEX, 100 µg/kg per day (solid bar) or EP80317 300 µg/kg per day (cross-hatched bar) on oxLDL (250 µg i.p., < 6 nmol MDA/mg lipoprotein)-induced accumulation of macrophages in the peritoneal cavity in wild type C57BL/6 mice and CD36 null mice fed a HFHC diet. Peritoneal macrophage accumulation in wild type mice tended to be reduced by 37%. B. Effects of 12 weeks treatment with 0.9% NaCl (open bar), HEX, 100 µg/kg per day (solid bar) or EP80317 300 µg/kg per day (cross-hatched bar) on oxLDL-induced accumulation of macrophages in the peritoneal cavity in ApoE null mice fed either a HFHC or a normal diet. Peritoneal macrophage accumulation was reduced by 39% in mice fed a normal diet.

Figure 4

GHRPs reduced oxLDL-induced expression of CD36 in mouse macrophages in ApoE null mice fed a HFHC diet for 12 weeks. Effects of 12 weeks treatment (from 6 weeks old) with 0.9% NaCl (open bar), HEX, 100 µg/kg per day (solid bar) or EP80317 300 µg/kg per day (cross-hatched bar) on oxLDL (250 µg i.p., < 6 nmol MDA/mg lipoprotein)-induced accumulation of macrophages in the peritoneal cavity in wild type C57BL/6 mice and CD36 null mice fed a HFHC diet. Peritoneal macrophage were harvested and 1×10^6 macrophages were assayed for CD36 protein level determination by Western blot. CD36 protein was reduced to 57 and 27% of controls, in macrophages from HEX and EP 80317-treated mice, respectively.

Figure 5

GHRPs did not modulate the growth curve in ApoE null mice fed a HFHC diet (or a normal diet) for 12 weeks. A. Effects of 12 weeks treatment (from 6 weeks old) with 0.9% NaCl (diamond), HEX, 100 µg/kg per day (square) or EP80317 300 µg/kg per day (triangle) on weight of in mice fed a HFHC or B, a normal diet.

Figure 6

GHRPs did not modulate food intake in ApoE null mice fed a HFHC diet (or a normal diet) for 12 weeks. A. Effects of 12 weeks treatment (from 6 weeks old) with 0.9% NaCl (diamond), HEX, 100 µg/kg per day (square) or EP80317 300 µg/kg per day (triangle) on food intake in mice fed a HFHC or B, a normal diet.

Figure 7

A. GHRPs reduce lipid accumulation in differentiated human macrophages. To induce lipid accumulation, human monocytic THP-1 cells were differentiated to macrophages with 5ng/ml PMA for 48hrs and then treated as indicated for 24hrs with 10uM HEX, or EP80317 in the presence of PMA. Non-differentiated cells (no PMA) were also analyzed. Lipid accumulation was quantified by photometry at 510nm following extraction of Oil red O from stained cells. Lipid staining was reduced by 35% and 28% in macrophages treated with HEX and EP80317, respectively. B. GHRPs reduced lipid accumulation in peritoneal macrophages isolated from ApoE null mice fed a HFHC diet from 13 weeks old. EP80317 (300 µg/kg) s.c. has been administered from 13 to 18 weeks old. Lipid staining (assessed by Oil red O extraction) was reduced by 57%.Figure 8

GHRPs increase expression of genes involved in cellular cholesterol removal.

A, PMA-differentiated THP-1 macrophages were treated with HEX or EP80317 as described in Figure 7, and mRNA expression was analyzed for selected genes by RT-PCR. The procedure involves extraction of total RNA from cells which is then reverse transcribed into cDNA and amplified in a PCR reaction with specific primers. Primers used are:

LXRα forward CCTGTCAGAAGAACAGATCCGC

LXRα reverse TCTTCAGCAGGGCAATCTGGTCC;

ABCA1 forward GGTCAATGGAAGGTTCAGGTGC

ABCA1 reverse GGAGTCGCTTTTTGCTCTGGGAGAGG;

GAPDH forward GGTCTTACTCCTTGGAGGCCATGT

GAPDH reverse GACCCCTTCATTGACCTCAACTACA.

B, Measurement of signal intensity from the experiment described in A was performed using an Alpha Imager analysis system and results expressed as fold response compared to untreated differentiated cells. GAPDH was used as a control for data normalization.

EXAMPLE 1

Growth hormone-releasing peptides prevent fatty streaks formation

Severe morbidity results as a consequence of the arterial disease atherosclerosis and complications of the disease, such as myocardial infarction and strokes, remain a common cause of mortality in Western Society (27). Current management strategies of atherosclerosis include life-style interventions such as healthy dietary and exercise recommendations and, mainly, the use of lipid lowering drug therapy, in addition to blood pressure control and use of antiplatelet drugs. Among hypolipemic drugs, hepatic hydroxymethylglutaryl-coenzyme A reductase inhibitors or statin drugs interfere with hepatic cholesterol metabolism which leads to a compensatory increase in LDL receptors and cholesterol clearance via these receptors. In contrast, fibrates and nicotinic acid mainly reduce circulating VLDL whereas bile acid binding resins interfere with bile acid reabsorption, thereby increasing fecal excretion of cholesterol. However, statins, used in monotherapy or in association with another lipid-lowering drug, have been associated with serious adverse effects, myopathy and rhabdomyolisis (28). For instance, cerivastatin therapy has been associated with 100s of myopathies and some dozens of deaths (29). Hence, use of these drugs should be revisited (30). In contrast, the mechanism of action of GHRPs, although not completely elucidated, is likely to involve the function of CD36 and its expression.

To determine whether GHRPs reduces atherosclerosis development, we have used the ApoE deficient mice strain and their C57BL/6 control littermates to assess the effects of prolonged (12 weeks) GHRPs treatment on fatty streak formation in mice fed a an enriched lipid diet. The surface area of oil red-O staining aortas of mice has been used as an index of plaque development and changes in the plasma levels of lipids as an index of hypolipemic effect. Taken together, these end points served to evaluate the anti-atherosclerotic effect GHRPs *in vivo*.

Methods

Drugs

HEX and EP80317 were a generous gift of Dr. R. Deghenghi, Europeptides, Argenteuil, France. HEX and EP80317 stock solutions were prepared in sterile 0.9% NaCl.

Animals

CD36-deficient and ApoE-deficient mice, as well as their control littermate were raised in the animal facilities of the Université de Montréal. The ApoE deficient mouse features the progressive series of atherogenic events seen in human, including increased adhesive interactions between leukocyte and endothelium, conversion of monocyte-

derived macrophages into foam cells with lesions distributing throughout the arterial tree, and late development of more advanced lesions (fibrous plaques). ApoE null mice show very high levels of plasma cholesterol as a result of impaired clearance of cholesterol-enriched lipoproteins and, as for humans, HCHF diet exacerbates disease progression and markedly enhances plasma cholesterol levels (7) (25). However, complex lesions as seen in humans are not observed except for the innominate artery in mice ~ 42 weeks old, where loss of continuity of the fibrous cap, rupture of xanthomas at the shoulders of lesions and intraplaque haemorrhage are seen (31). Evidence accumulates to support that fatty streaks in anatomical sites prone to atheromatous plaque development precede mature lesions in humans, although all fatty streak do not progress to atheromas (26). In both human and mice, T lymphocyte and foam cells are found in fatty streaks and immunological processes have been shown to be similar over the years in both species.

The animals were housed in cages (less than 5 per cage) and fed a normal chow diet and water ad libitum. Male C57BL/6, CD36 and ApoE null mice were assigned to 1 of 3 groups (n=12 mice per group): Group 1 received daily injections of HEX (100 µg/kg, 1 µl/g); group 2, EP 80 317 (300 µg/kg, 1 µl/g), and group 3, 0.9% NaCl. Daily s.c. treatment with HEX, EP80317 or vehicle was begun at 6 weeks old and continued for 12 weeks. Four days before sacrifice, 6 mice from groups 1 to 3 were injected i.p. with oxLDL (minimally oxidized, TBARS 3-6 nmol/mg protein). At 18 weeks old, mice were fasted overnight, anesthetized with ketamine-xylazine (90:10 mg/kg) two hours after the s.c. administration of the morning dose of the drug under study. Blood (1 ml) was taken from the heart and put into EDTA pre-coated microcontainers (BD, Franklin Lakes, NJ, USA). Mice were killed, the hearts were perfused with 20 ml 0.9% NaCl. The peritoneal cavity was washed with 3 ml of heparinized saline (10 units/ml) in mice injected i.p. with oxLDL. A hemacytometer and stained cytospin preparation (Diff Quick stain, Dade Diagnostics of P.R. Inc., Aguada, PR) were used to determine the total and differential leukocyte numbers, respectively, for the peritoneal cavity lavage fluid. The entire aorta from the heart, extending 5-10 mm after bifurcation of the iliac arteries and including the subclavian right and left common carotid arteries, was removed, dissected, and evaluated for lesion development by en face oil red-O staining and morphometry of scanned images using the software Scion Image (Scion Corp., Fredrick, Maryland). The animal study protocol was reviewed and approved by the institutional Animal Ethics Committee of the Université de Montréal and conducted in accordance with the Canadian Council on Animal Care guidelines for use of experimental animals.

Plasma lipid analysis

Total plasma cholesterol, triacylglycerol and HDL cholesterol were determined using enzymatic kits (Sigma Chemicals). Appropriate standards and controls were included in each assay.

Statistical analysis

Data are expressed as mean ± SEM. Comparisons between groups were performed using a one-way analysis of variance (ANOVA) followed by pair-wise multiple comparisons using the Student-Newman-Keuls method. Differences were considered significant at $p < 0.05$.

Results

The most striking observation is a reduction of lesions area in ApoE-deficient mice, by 28 and 47% following treatment with HEX (100 µg/kg) and EP80317 (300 µg/kg) daily, respectively, in mice fed a HFHC diet (fig 1). CD36-deficient mice and their wild type C57BL/6 control littermates did not develop significant fatty streak lesions on HFHC diet (12 weeks).

Reduced lesions area was accompanied with a decrease in total plasma cholesterol (30%), as well as in non HDL plasma cholesterol (31%), in ApoE-deficient mice fed a HFHC diet and treated daily with EP80317 (300 µg/kg) daily for 12 weeks, as compared to controls and to ApoE null mice treated treated with HEX (100 µg/kg) daily (Fig 2A and Fig 2C). In contrast, HDL cholesterol tended to be increased in ApoE null mice under lipid diet (12 weeks) treated with HEX (65%) or EP80317 (73%) as compared to controls on HFHC diet (Fig 2D). Plasma triglycerides did not change significantly (Fig 2B). GHRPs did not modulate total plasma cholesterol levels in CD36-deficient or C57BL/6 controls fed a HFHC diet (data not shown).

EP80317 reduced oxLDL-induced peritoneal macrophage accumulation by 37% and 39 % in wild type C57BL/6 and ApoE null mice, respectively (Fig 3A and Fig. 3B). EP 80317 did not modulate macrophage accumulation in CD36 null mice (Fig 3A). CD36 protein expression was reduced to 57 and 27% of controls, in peritoneal macrophages harvested from HEX and EP 80317-treated ApoE null mice fed a HFHC diet, respectively (Fig. 4).

GHRPs therapy did not affect the growth curve in ApoE-deficient mice fed a HFHC diet (Fig 5A) or a normal diet (Fig 5B), nor the food intake in these mice (Fig 6A and Fig 6B).

Discussion

In the present study, we have assessed the contribution of GHRPs in the protective effect against atherosclerosis development. The major findings are 1) that a prolonged treatment with GHRPs protects mice fed a HFHC diet from developing fatty

streak lesions 2) these protective effects of GHRPs are associated with a favourable modulation in plasma lipids, inasmuch as total plasma cholesterol and non HDL cholesterol are reduced, and HDL plasma cholesterol is increased, in ApoE null mice fed a HFHC diet 3) the atheroprotective effects of GHRPs are associated with a reduced expression of CD36 protein in macrophages. These findings suggest the possibility that GHRPs therapy, through reducing atherosclerosis development, may afford protection against heart attacks and strokes. Thus, GHRP and their synthetic analogs would appear to be potentially helpful for the treatment or prophylaxis of coronary cardiovascular diseases. For example, they will be useful to prevent hypercholesterolemia and atherosclerosis, thereby reducing the complications associated with the disease. Currently, only few drugs have the capacity to achieve these goals, and these have been associated with potentially severe adverse effects.

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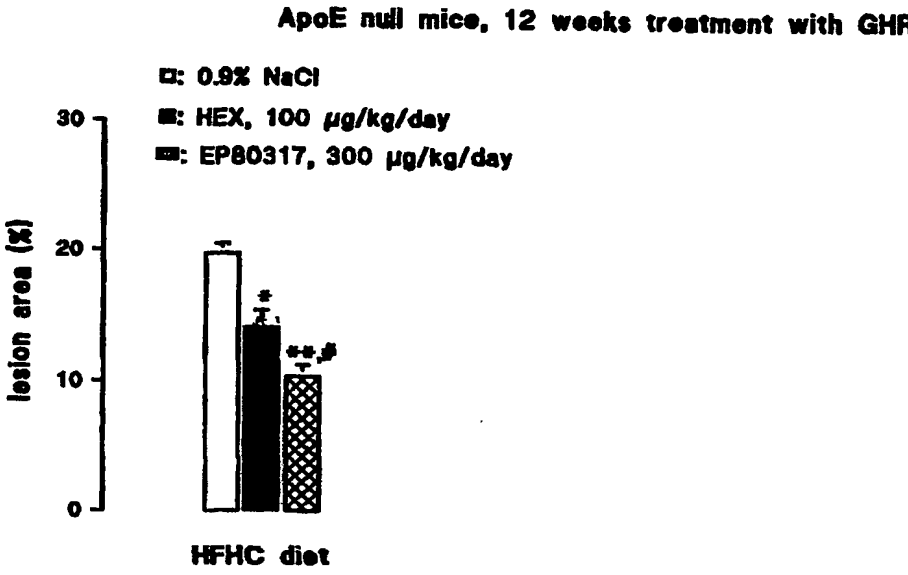
1. The use of growth hormone releasing peptides of Hexarelin family, of derived peptidomimetics and of CD36 ligands in the prevention and treatment of atherosclerosis and hypercholesterolemia.

2. The use of GHRP derivatives, of derived peptidomimetics, and of CD36 ligands which modulate the expression of scavenger receptor B (CD36) in the development of atherosclerotic lesions and in the prevention of heart attacks and strokes associated with coronary artery disease and hypercholesterolemia.

3. The use of GHRP derivatives and of derived peptidomimetics which modulate the expression of the ATP-binding cassette ABCA1 transporter scavenger receptor B (CD36) in the development of atherosclerotic lesions and in the prevention of heart attacks and strokes associated with coronary artery disease and hypercholesterolemia.

4. A pharmaceutical composition containing a compound as claimed in claims 1, 2 and 3, to be administered exogenously.



*, $p < 0.01$ and **, $p < 0.001$ compared with 0.9% NaCl; #, $p < 0.05$ compared with HEX

Figure 1A

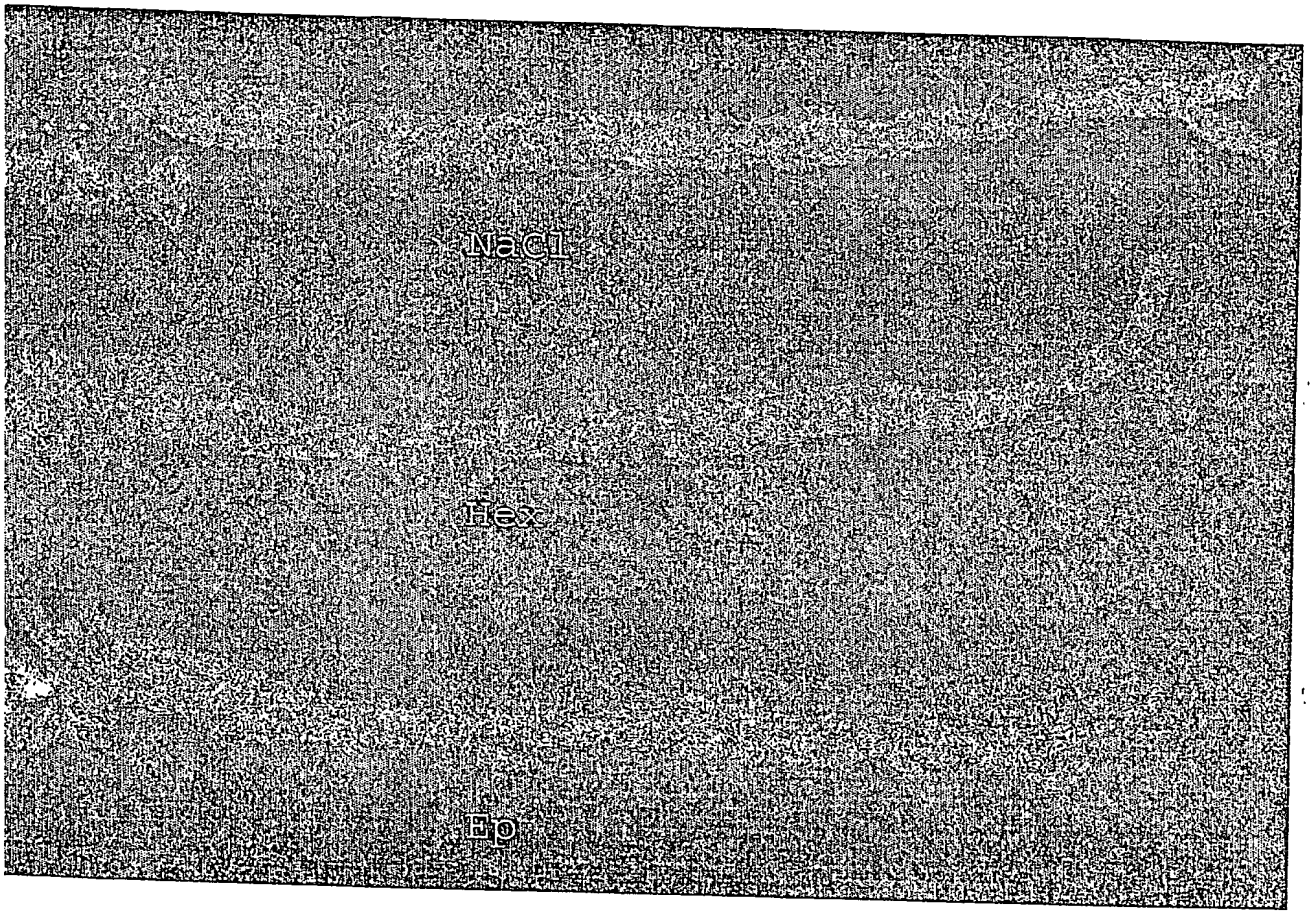
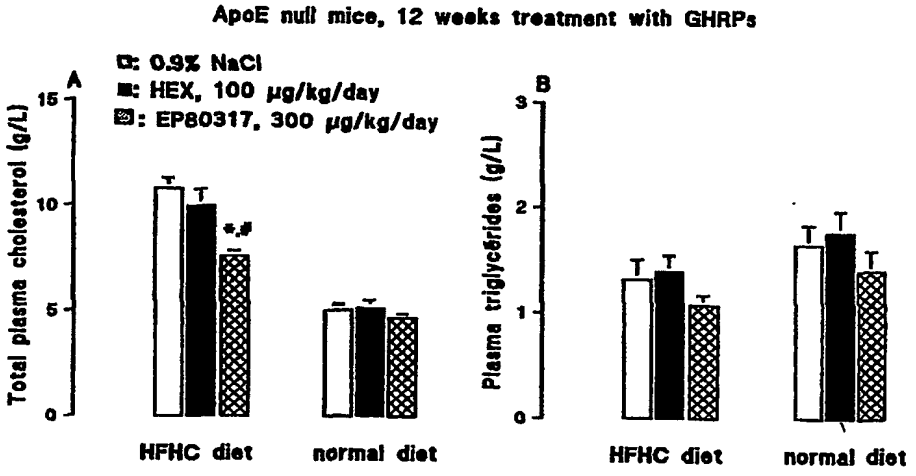
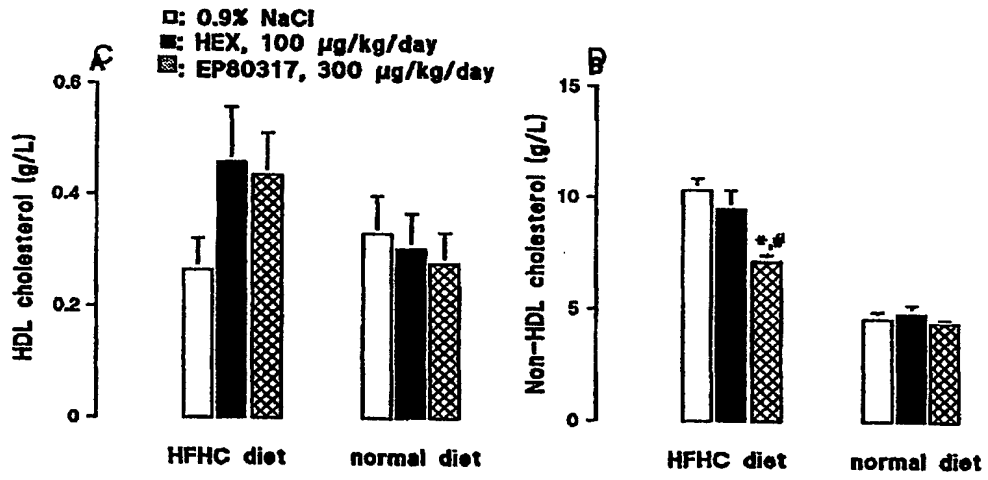


FIGURE 1B



*, p < 0.01 compared with 0.9% NaCl; #, p < 0.01 compared with HEX



*, p < 0.01 compared with 0.9% NaCl; #, p < 0.01 compared with HEX

Figure 2

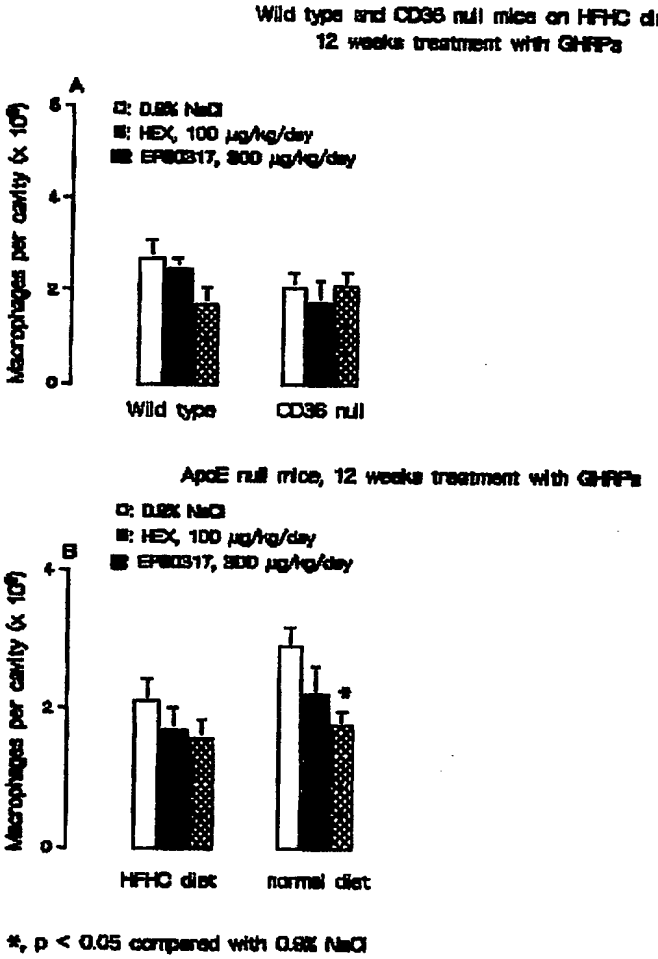

Wild type and CD36 null mice on HFHC diet
12 weeks treatment with GHRPs
A
Macrophages per cavity (x 10⁶)
6
4
2
0
□: 0.9% NaCl
▥: HEX, 100 µg/kg/day
■: EP80317, 300 µg/kg/day
Wild type
CD36 null
ApoE null mice, 12 weeks treatment with GHRPs
B
Macrophages per cavity (x 10⁶)
4
2
0
□: 0.9% NaCl
▥: HEX, 100 µg/kg/day
■: EP80317, 300 µg/kg/day
*
HFHC diet
normal diet
*, p < 0.05 compared with 0.9% NaCl

Figure 3

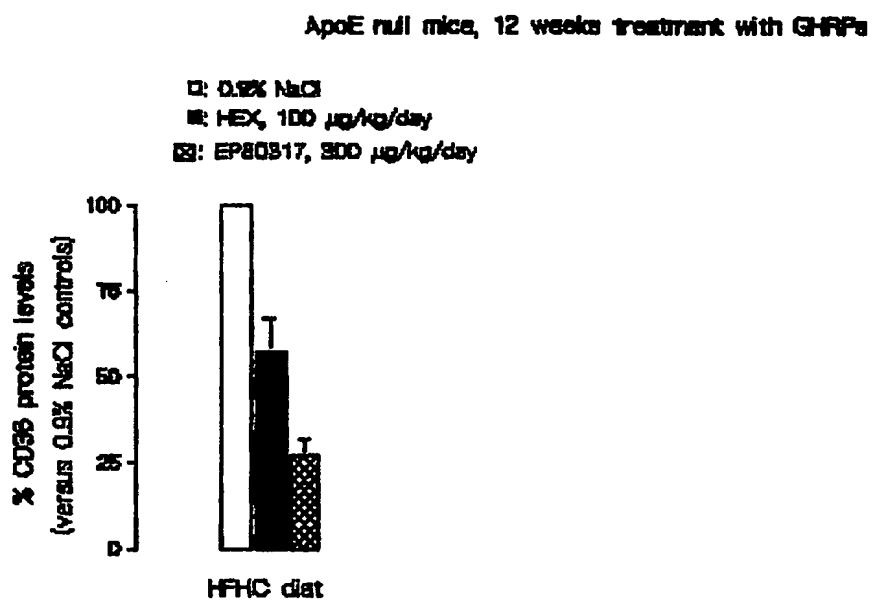
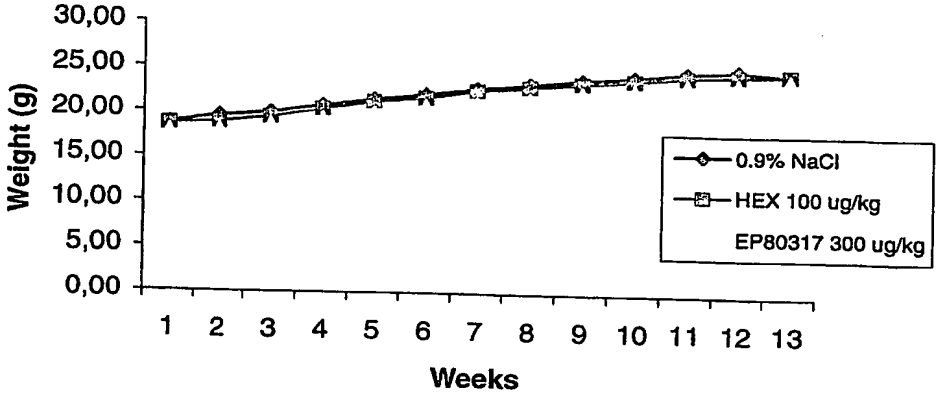

ApoE null mice, 12 weeks treatment with GHRPs
□: 0.9% NaCl
■: HEX, 100 µg/kg/day
⊠: EP80317, 300 µg/kg/day
% CD36 protein levels
(versus 0.9% NaCl controls)
100
75
50
25
0
HFHC diet

Figure 4

A. Variation in weight of ApoE null mice on lipid diet



B. Variation in weight of ApoE null mice on normal diet

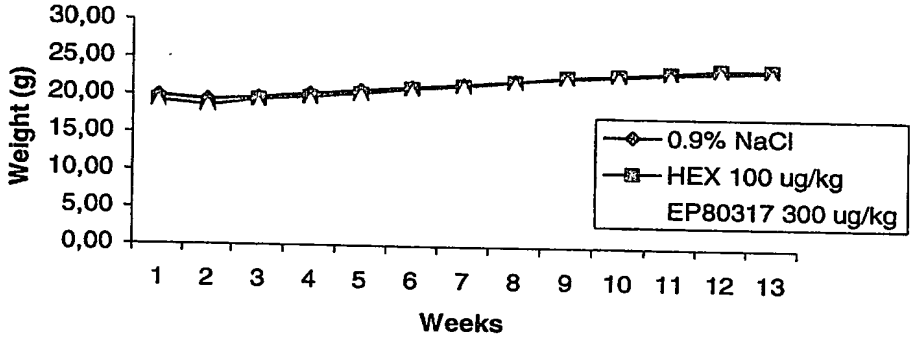
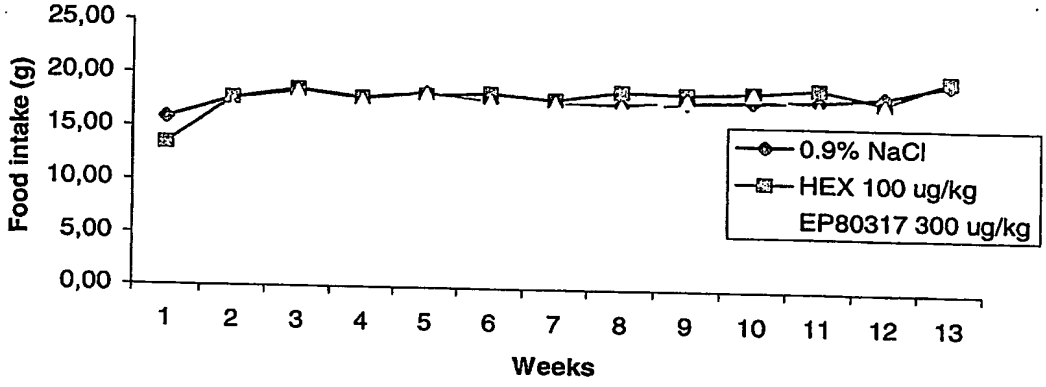


Figure 5

A. Food intake per week in ApoE null mice on lipid diet



B. Food intake per week in ApoE null mice on normal diet

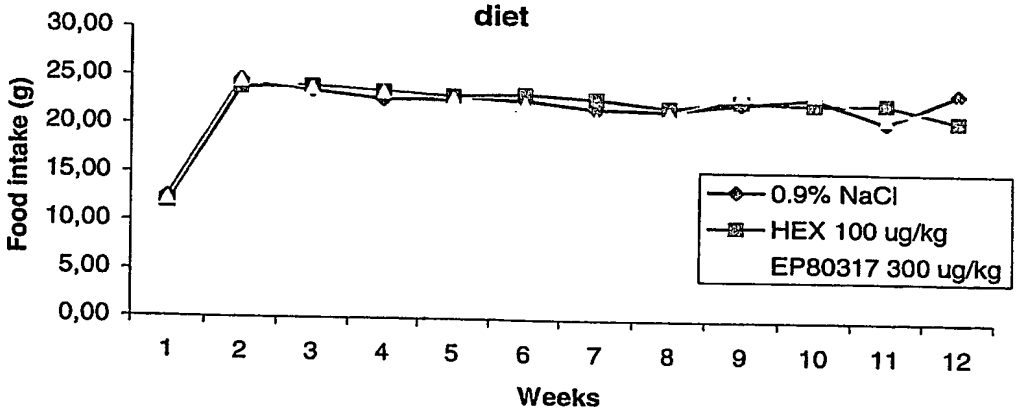


Figure 6

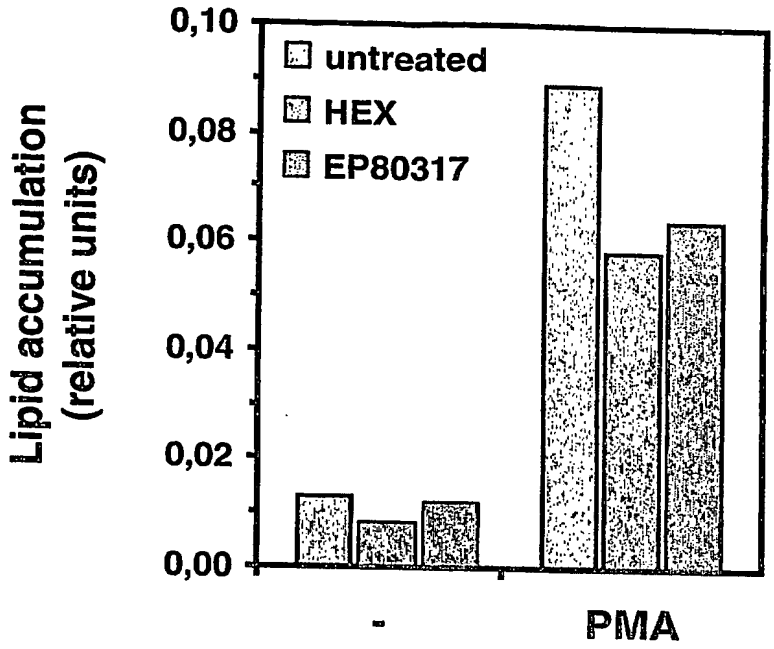


Figure 7A

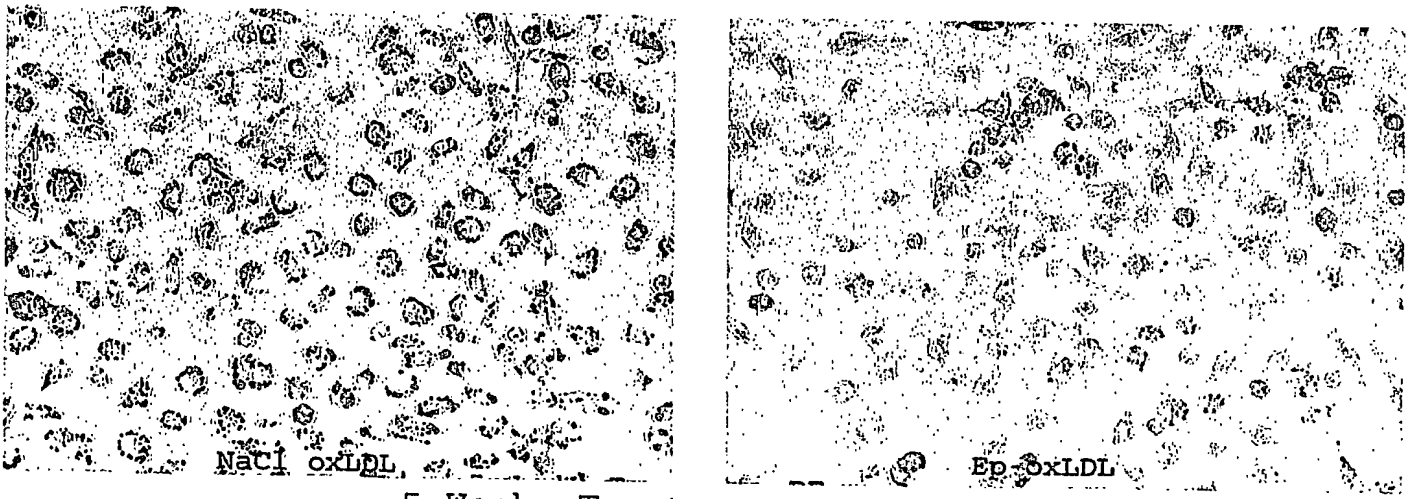


Figure 7B

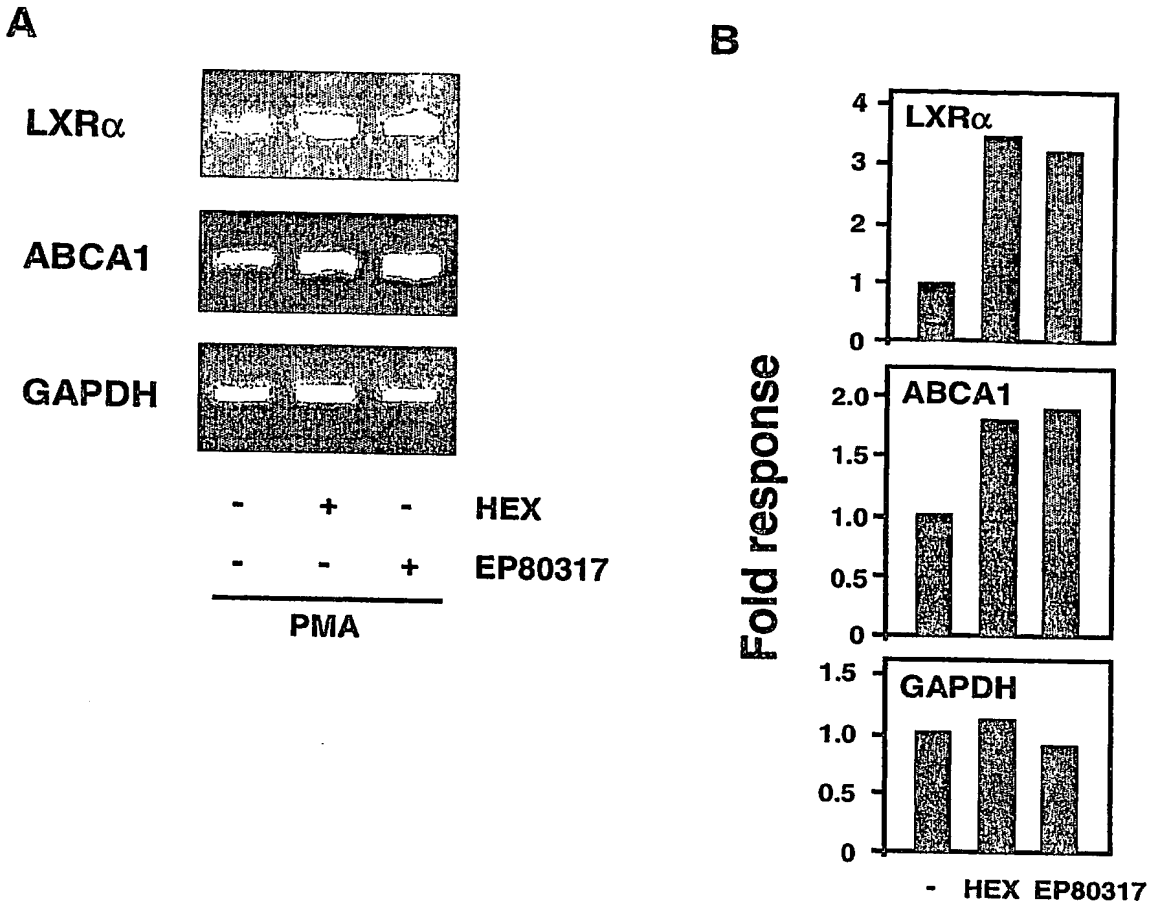

A
LXRα
ABCA1
GAPDH
- + - HEX
- - + EP80317
PMA
B
Fold response
LXRα
4
3
2
1
0
ABCA1
2.0
1.5
1.0
0.5
0
GAPDH
1.5
1.0
0.5
0
- HEX EP80317

Figure 8